Immunology
The Journal of cells, molecules, systems and technologies

British Society for immunology

IMMUNOLOGY REVIEW ARTICLE

Endocannabinoid signalling in innate and adaptive immunity

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doi:10.1111/imm.12441
Received 28 October 2014; revised 23 December 2014; accepted 5 January 2015.
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Summary

The immune system can be modulated and regulated not only by foreign antigens but also by other humoral factors and metabolic products, which are able to affect several quantitative and qualitative aspects of immunity. Among these, endocannabinoids are a group of bioactive lipids that might serve as secondary modulators, which when mobilized coincident with or shortly after first-line immune modulators, increase or decrease many immune functions. Most immune cells express these bioactive lipids, together with their set of receptors and of enzymes regulating their synthesis and degradation. In this review, a synopsis of the manifold immunomodulatory effects of endocannabinoids and their signalling in the different cell populations of innate and adaptive immunity is appointed, with a particular distinction between mice and human immune system compartments.

Keywords: cell signalling; endocannabinoids; immune cells.

The endocannabinoid system

Although Δ^9-tetrahydrocannabinol was isolated exactly 50 years ago, it was only at the beginning of the 1990s that cannabinoid receptors were described and cloned in the brain, so explaining why our body reacts to cannabis extracts and representing the first evidence for the presence of the so-called 'endocannabinoid system' (ECS). The discovery of cannabinoid receptors initiated a quest for their endogenous ligands, which progressively led to the identification and isolation of a new family of *N*- or *O*-derivatives of polyunsaturated fatty acids able to act as cannabinoid receptor agonists and collectively termed endocannabinoids (eCBs).[1,2]

The endocannabinoids and their metabolism

Endocannabinoids include a group of lipid mediators, of which the best characterized members are *N*-arachidonoylethanolamine (anandamide, AEA) and 2-arachidonoylglycerol (2-AG).[3,4] Some other compounds have been proposed to belong to the eCB family, including 2-AG-ether (noladin ether) and *O*-arachidonoylethanolamine (virodhamine).[2] Among these 'eCB-like' compounds, two additional *N*-acylethanolamines, namely *N*-palmitoylethanolamine (PEA) and *N*-oleoylethanolamine, have been extensively investigated because of their anti-inflammatory and analgesic properties,[5–7] and anorexic effects, respectively.[8] Endocannabinoids are synthesized and released 'on demand' (if and when needed) from membrane phospholipids in response to physiological or pathological stimuli. However, this 'dogma' has been lately reconsidered because of the discovery of intracellular transporters and storage organelles/pools that might serve as potential platforms for eCB trafficking and accumulation. This novel concept adds more complexity to eCB homeostasis and certainly makes them more available both for receptor activation and for distinct metabolic pathways, away from the site and time of their biosynthesis.[9–12] Biosynthesis of AEA and of its congeners includes two steps: *N*-arachidonoyl-phosphatidylethanolamine is formed from phosphatidylethanolamine by calcium-dependent *N*-acyl-transferase, and is then converted through at least five distinct metabolic pathways into AEA or other *N*-acylethanolamines.[13] The most studied route for such a conversion involves the *N*-acyl-phosphatidylethanolamine-hydrolysing phospholipase D,[14] but other alternative yet relevant pathways engage phospholipase A and

lyso-phospholipase D,[15] α/β-hydrolase 4 and glycerophosphodiesterase 1,[16] or phospholipase C and protein tyrosine phosphatase type-22.[17] The biosynthesis of 2-AG starts from *sn*-1-acyl-2-arachidonoylglycerols (DAGs), that can be directly converted into 2-AG through the action of two Ca^{2+}-sensitive *sn*-2-selective DAG lipases, i.e. DAGL-α and DAGL-β.[18] A less-characterized pathway for 2-AG biosynthesis involves the generation of 2-AG-3-phosphate, which is a lysophosphatidic acid.[19] All eCBs are then inactivated by a two-step process: cellular uptake through a purported 'endocannabinoid membrane transporter', whose molecular identity has yet to be identified, and intracellular hydrolysis. AEA is principally cleaved by fatty acid amide hydrolase (FAAH) into arachidonic acid and ethanolamine,[20] but also by another enzyme, *N*-acylethanolamine-hydrolysing acid amidase, which is mainly involved in the hydrolysis of PEA and whose physiological implications are still unclear.[13] 2-AG can be cleaved into glycerol and arachidonic acid by FAAH, though its main hydrolase is a monoacylglycerol lipase, responsible for ~ 85% of 2-AG hydrolysis in the mouse brain.[21,22] In addition, 2-AG can also be cleaved by two integral membrane proteins, α/β-hydrolase domain-containing proteins 6 and 12.[21,22] Furthermore, AEA and 2-AG are substrates of cyclooxygenase-2 (COX-2), different lipoxygenase isozymes and cytochrome P450, leading to oxidized compounds like prostaglandin-ethanolamides and -glyceryl esters, hydroxy-anandamides and hydroxyeicosatetraenoyl-glycerols, respectively, all endowed with distinct biological activities.[22] The main elements of the ECS are schematically depicted in Fig. 1.

Molecular targets and signalling pathways

Once synthesized, eCBs bind to and functionally activate their target receptors, triggering various signalling pathways and causing several biological effects on different tissues (Fig. 1). The main receptor targets for eCBs are type-1 (CB_1) and type-2 (CB_2) G protein-coupled cannabinoid receptors.[23] CB_1 is widely expressed in the nervous system, mainly at the terminal ends of central and peripheral neurons, and its presence has also been widely documented in many different extraneural sites. Once activated, CB_1 is involved in the inhibition of excitatory and inhibitory neurotransmission and can modulate cognitive, memory and motor functions, as well as analgesia. CB_2 is mainly expressed by cells of the immune system where it is commonly associated with the regulation of different immune functions.[24] The identification of CB_2 in brainstem neurons and its presence in activated microglial cells and astrocytes, or in certain subsets of neurons upon insult,[25,26] has led to an 'identity crisis' of this receptor.[27] Indeed, the up-regulation of CB_2 is associated with chronic inflammation of the nervous system, as well as with several cardiovascular and bone disorders.[28,29] CB_1 and CB_2 are metabotropic receptors that usually couple to heterotrimeric Gi alpha subunit proteins, and so trigger the canonical signalling pathway of inhibition of adenylyl cyclase activity and reduction of cAMP levels, which lead to the inactivation of protein kinase A. CB_1 and CB_2 also activate various effector protein kinase cascades involved in cell proliferation and survival; these include the phosphatidylinositol 3-kinase/protein kinase B, the mitogen-activated protein kinase p38, the extracellular-signalling regulated protein kinase-mitogen-activated protein kinase, as well as the focal adhesion kinase.[29] Other signalling pathways include coupling to ion channels (N- and P/Q-type Ca^{2+} channels and voltage-gated K^+ channels), activation of phospholipase-Cβ, and ceramide biosynthesis.[29] In addition to CB_1 and CB_2, it is now clearly established that eCBs can engage other non-CB targets.[23] The best known is the transient receptor potential vanilloid 1 channel, activated intracellularly by AEA and 2-AG,[30,31] which is expressed in sensory neurons and in epithelial, endothelial and immune cells.[32] Also peroxisome proliferator-activated receptor (PPAR) α and γ,[33] which belong to a family of nuclear receptors able to alter lipid turnover and metabolism, as well as the orphan G protein-coupled receptor GPR55,[34] are activated by eCBs. Probably these additional targets call for reconsideration of the name 'cannabinoid receptor', which might be readapted to take into consideration all the molecular targets identified so far for eCBs.

Role of ECS in the regulation of immune responses

Over the last 20 years, the ECS has been thoroughly studied in most cell types and tissues. Its role in the regulation of the immune system is probably the most flourishing and promising, mostly due to the increasing recognition of the eCBs signalling in several chronic inflammatory diseases. Also the fact that essentially all immune cells secrete eCBs, are capable of regulating their synthesis and degradation and possess cannabinoid receptors supports this view.[35–38] It is now generally accepted that the immunosuppressive effects of eCBs on immune cells are primarily mediated through CB_2, whose expression is usually higher than that of CB_1.[28,39] Unlike eCBs and their metabolizing enzymes, the presence and distribution of cannabinoid receptors within immune cells strongly vary and have been mainly investigated in human immune cell populations.[40,41] Very few studies have addressed the differential expression of cannabinoid receptors on mouse immune cell subsets.[42] Recently, a detailed analysis of CB_2 protein levels expressed by the various blood immune cells from healthy human donors revealed that natural killer (NK) cells, B lymphocytes and monocytes express a higher level of CB_2 than $CD4^+$ or $CD8^+$ T lymphocytes or neutrophils. However, NK cells have the greatest variation in CB_2

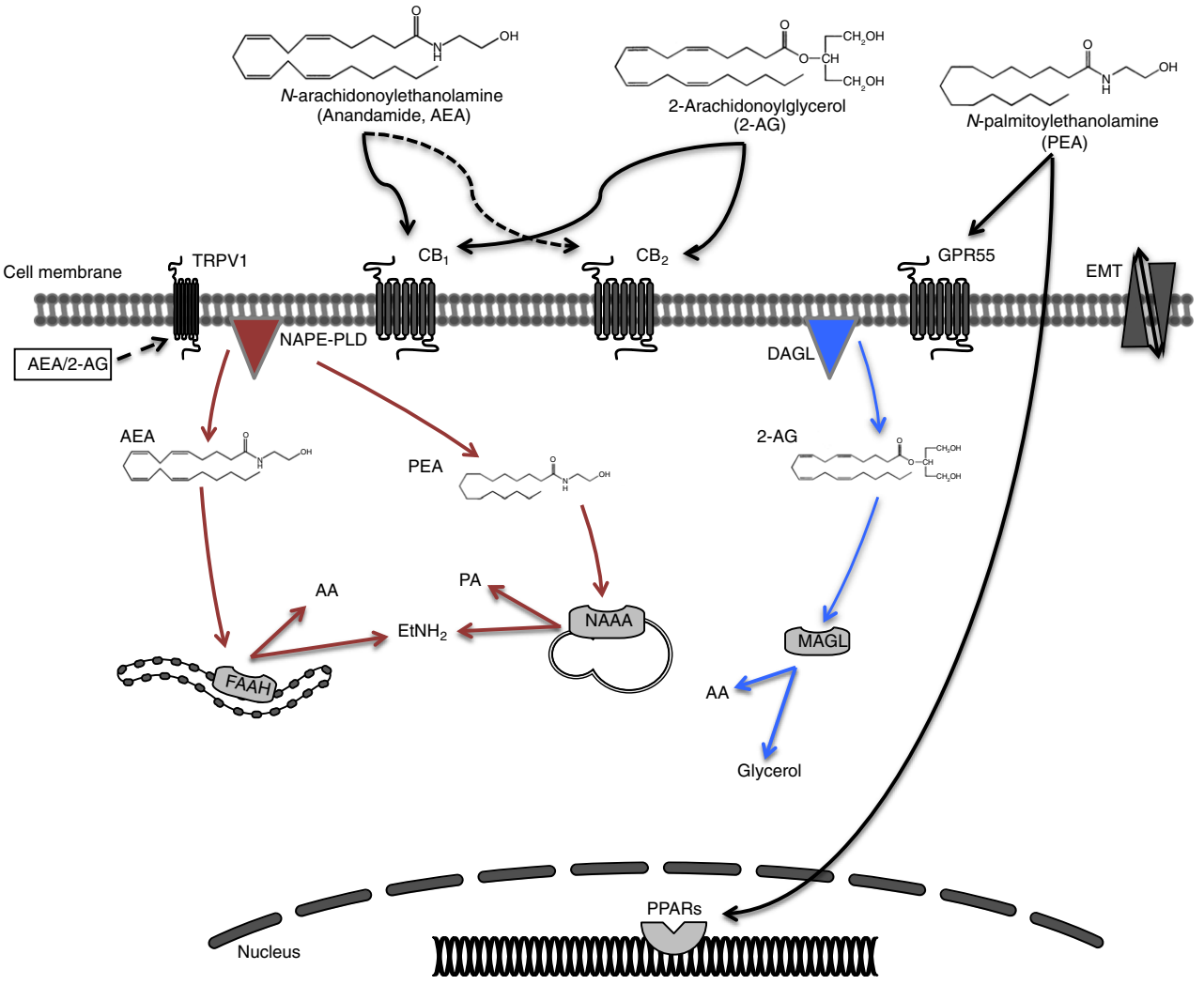

Figure 1. Metabolism of the main immunoregulatory endocannabinoids (eCBs). *N*-Arachidonoylethanolamine (AEA) or *N*-palmitoylethanolamine (PEA) and 2-arachidonoylglycerol (2-AG) are usually released on demand from membrane lipids, through the activity of *N*-acyl-phosphatidylethanolamine-hydrolysing phospholipase D (NAPE-PLD) and *sn*-1-acyl-2-arachidonoylglycerol lipase (DAGL), respectively and move across the plasma membrane via a purported endocannabinoid membrane transporter (EMT). Targets of AEA and 2-AG are CB_1 and CB_2, which show an extracellular binding site. AEA also binds to transient receptor potential vanilloid 1 (TRPV1), which bears an intracellular binding site. PEA binds and activates peroxisome proliferator-activated receptors (PPARs) and G protein-coupled receptor 55 (GPR55). Dashed lines represent low-affinity bindings. Once eCBs bind to their target receptors, different signalling pathways can be activated depending on the cellular environment (see text for detail). After their actions, eCBs are taken up by EMT for inactivation; AEA is hydrolysed by fatty acid amide hydrolase (FAAH) to ethanolamine and arachidonic acid, 2-AG is hydrolysed by monoacylglycerol lipase (MAGL) and to a minor extent by FAAH, releasing glycerol and arachidonic acid and PEA is hydrolysed by *N*-acylethanolamine-hydrolysing acid amidase (NAAA) into ethanolamine and palmitic acid.

expression levels, whereas for each of the other cell types CB_2 levels are relatively similar between subjects.[43] The low abundance of CB_2 on resting T lymphocytes significantly increases on activated $CD4^+$ and $CD8^+$ human T cells. The current view is that eCBs, rather than just exerting either immunosuppressive or stimulatory effects on the immune system, are more likely to be part of a homeostatic immunoregulatory scheme. The majority of scientific studies on the immunoregulatory role of eCBs concentrated on whole immune cells, either on peripheral blood mononuclear cells or on mouse splenocytes, where AEA and PEA are mostly anti-inflammatory,[44–46] and 2-AG exerts both pro-inflammatory and anti-inflammatory effects.[47–50] Therefore, in this section we will address the immunoregulatory functions of the main eCBs and their signalling on the different immune cell populations of both innate and adaptive immunity, devoting special attention to whether they stem from peripheral human or murine immune cells, be they immortalized cell lines or primary cells. Indeed, mice are the most frequently used

animal and the experimental tool of choice for the majority of immunologists. Study of their immune responses has yielded tremendous insight into the workings of the human immune system.[51] However, a thorough demarcation is sought, not only because relevant differences exist between the immune system of humans and mice, but also because immortalized cell lines often respond differently from primary cells.

Endocannabinoid signalling in innate immunity

Monocytes/macrophages

Macrophages (and their precursors, monocytes) play an important role in innate immunity, because they not only clear apoptotic cells and pathogens, but also instruct other immune cells. Monocytes/macrophages are highly plastic (they can change their functional phenotype depending on environmental cues) and reside in every tissue of the body, where they bear different names (i.e., Kupffer cells in the liver or microglia in the central nervous system).[52] CB_1 and CB_2 receptors are highly expressed in both murine and human monocytes/macrophages and microglial cells, regardless of cellular models.[41,42,53–57] Similarly, all eCB metabolic enzymes are often modulated in response to inflammatory stimuli, so regulating eCBs tone *in vivo*.[58–61] Interestingly, a recent study reported the existence of bidirectional eCB transport across cell membranes of a monocytic cell line, combining both radioligand assays and quantification of intracellular and extracellular levels of AEA and 2-AG upon differential pharmacological blockage of their uptake, breakdown and interaction with binding proteins.[62] These data extend a previous report on the in and out transport of AEA across human umbilical vein endothelial cells.[63] The first evidence of an immunoregulatory role of eCBs on monocytes/macrophages came from a study on mouse alveolar macrophages, where AEA inhibited macrophage-mediated killing of tumour necrosis factor-sensitive cells.[64] Later evidence supported the anti-inflammatory nature of AEA, according to which this endogenous lipid inhibited the expression of pro-inflammatory mediators such as nitric oxide and interleukins IL-6, IL-12 and IL-23, and enhanced anti-inflammatory mediators like IL-10 and CD200R. Nonetheless, these overt immunosuppressive effects were only seen in mouse macrophage cell lines and microglia[60,65–69] and in most cases were mediated by CB_2 signalling, whose involvement was also directly implicated in dectin-1-mediated phagocytosis.[70] Also, PEA exerts anti-inflammatory properties on murine microglia, mainly by stimulating phagocytosis and clearance of pathogens, and by increasing resistance to infection and microglial cell motility.[71–74] Conversely, there are scarce and contradictory data on the role of 2-AG in the modulation of mouse macrophage/microglia responses: on the one hand, 2-AG inhibits tumour necrosis factor-α (TNF-α) and IL-6 production and promotes alternatively activated and anti-inflammatory M2 macrophages;[60,75,76] on the other hand, it increases inducible nitric oxide synthase-dependent nitric oxide production.[60] Likewise, also in humans, 2-AG shows opposite effects. Indeed, it enhances the production of chemokines,[76] migration and adhesion of macrophage-like differentiated human HL-60, U937 and THP-1 cell lines, as well as peripheral blood monocytes in a CB_2-phosphatidylinositol 3-kinase-dependent pathway.[77–79] Yet, 2-AG was also reported to enhance the phagocytosis of opsonized zymosan in the same cell lines,[80] and to induce human monocytes to produce decreased levels of cytokines and adhesion molecules, thereby exhibiting an immunosuppressive response.[47] In some cases, it was not entirely clear whether the effects of 2-AG were actually mediated via CB_2 receptors. Incidentally, it has been suggested that discrepancies on the effects of 2-AG, and to a certain extent of AEA, could be due to their conversion into bioactive COX-2 metabolites.[81] A summary of the main effects on mouse and human monocytes/macrophages is shown in Fig. 2.

Dendritic cells

Dendritic cells (DCs) are the most professional antigen-presenting cells, crucial in the development of antigen-specific T-cell responses. They are present in those tissues that are in contact with the external environment, such as the skin (i.e. Langerhans cells), and the inner lining of several organs; they can also be found in peripheral blood (i.e. myeloid and plasmacytoid DCs).[82] Despite their role in shaping the type and quality of immune responses, due to their position at the crossroads between innate and adaptive immunity, very few studies have investigated endocannabinoid signalling in these cells, especially in humans (Fig. 3). A pioneering study came from Di Marzo and coworkers, who demonstrated for the first time the presence of the ECS (AEA, 2-AG, PEA, CB_1, CB_2 and FAAH) in human blood monocyte-derived DCs, and its regulation upon cell activation. In particular, although the expression of CB_1 and CB_2 remained unmodified following cell maturation induced by lipopolysaccharide or by the allergen *Der p* I, the levels of 2-AG (but not those of AEA or PEA) were significantly increased.[83] Following this first evidence, so far only one other study attempted to investigate the role of 2-AG in DCs, showing that it acts as a chemoattractant for both immature and mature bone marrow-derived mouse DCs. Additionally, 2-AG *in vivo* shifts the memory response towards the T helper type 1 (Th1) type.[84] At the same time it was found that high (micromolar) doses of AEA induce apoptosis in murine bone marrow-derived DCs, through both CB_1 and CB_2 receptors, providing a potential mechanism for

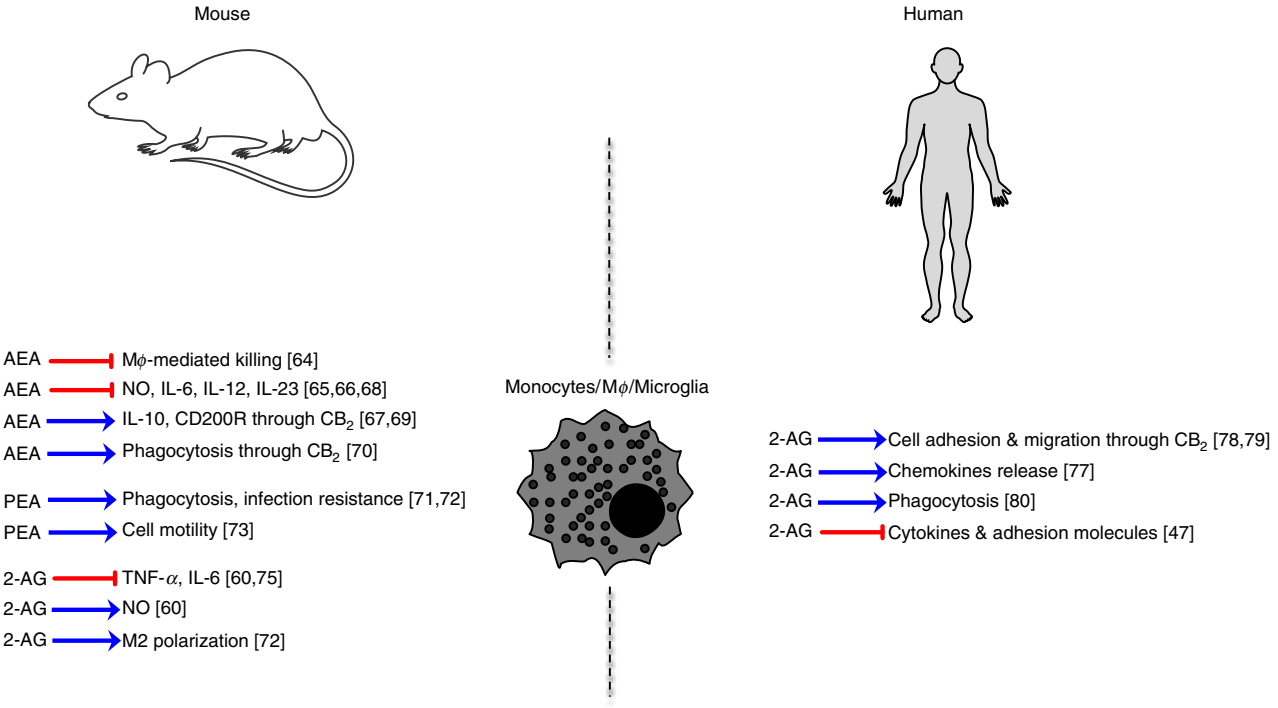

Figure 2. Schematic representation of endocannabinoid (eCB) signalling in murine and human monocytes/macrophages or migroglia. Mϕ, macrophages.

eCB-mediated immunosuppression of immune cells.[85] Interestingly, the efficacy of AEA depended on its rapid hydrolysis by FAAH, because pharmacological inhibition of the latter led to a reduced resistance to apoptosis. The involvement of CB_1 and CB_2 in determining DC responses was clearly elucidated by analysing the phenotypic and functional profile of murine bone marrow-derived DCs from $CB_1^{-/-}$ $CB_2^{-/-}$ mice. Indeed, deletion of both cannabinoid receptors exacerbated DC function by increasing their activation markers (MHC-I/II, CD80, CD86) leading to a more mature phenotype, as well as by eliciting a more robust T-cell response.[86] In contrast, it was reported that nanomolar and low micromolar doses of AEA before sensitization increased both the expression of murine DC co-stimulatory molecules (CD80/CD86) and IL-12/IL-23 production *ex vivo*.[87] Yet, identification of these DC was somehow imprecise, because their immunophenotypic profile was carried out in total splenocytes stained only with CD11c, a marker shared also by other cell types. The only additional evidence on human DCs was obtained by our group on circulating peripheral blood myeloid and plasmacytoid DCs. Notably, we found that low micromolar doses of AEA significantly inhibited TNF-α, IL-12p40 and IL-6, as well as TNF-α and interferon-α, from activated myeloid and plasmacytoid DCs via CB_2 respectively.[88] Furthermore, such an AEA-mediated immunosuppression of both DC subsets was also paralleled by a reduced ability of myeloid and plasmacytoid DCs to polarize naive CD4 T cells into Th1 and Th17 lineages.[88]

Neutrophils and NK cells

Neutrophils and NK cells are crucial cells of innate immunity, and are both involved in host defence against cancer and anti-microbial responses. Neutrophils are the first inflammatory cells to be recruited at the site of inflammation/injury and are the hallmark of acute inflammation, whereas NK cells are a type of cytotoxic lymphocyte that provide rapid responses against virally infected cells and cancer cells.[89,90] Surprisingly, although NK cells have been shown to express both CB_1 and CB_2 and to release high levels of AEA and 2-AG,[54] knowledge of eCB signalling in NK cells is almost null and is summarized in Fig. 4. Indeed, only two reports addressed the role of 2-AG in inducing the migration of the NK-differentiated human HL-60 cell line through CB_2 receptor.[91,92] This lack of evidence is probably a result of the difficulty in documenting cannabinoid receptors in NK cells, that show the greatest variation of expression of these receptors. However, our group has recently reported evidence that human peripheral blood NK cells express high levels of putative 'CB_3' receptor, whose activation enhances NK cell functions in terms of TNF-α and interferon-γ production, and of CD107a-mediated cell killing.[93] Instead, a great deal of information has been accumulated on the

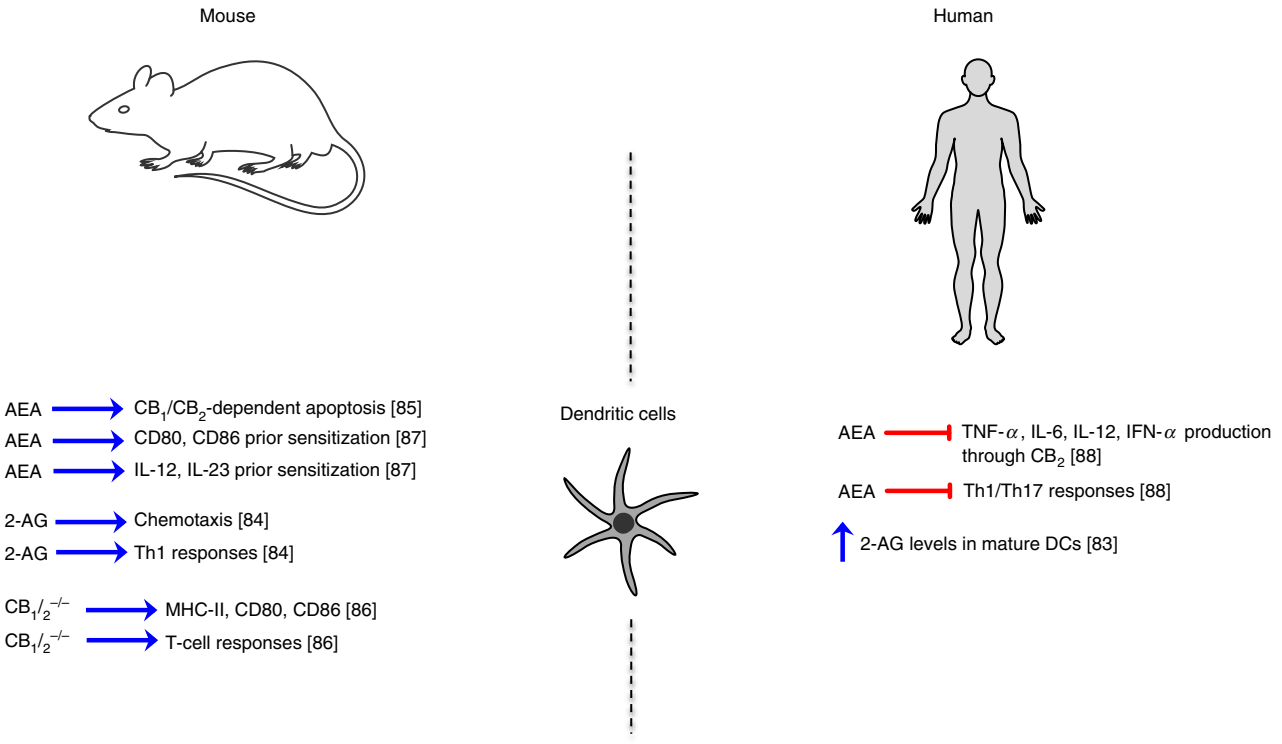

Figure 3. Schematic representation of endocannabinoid (eCB) signalling in murine and human dendritic cells.

role of both AEA and 2-AG signalling on neutrophils in humans (Fig. 4). Perhaps because of their abundance in peripheral blood, many studies have been performed on neutrophils. Although AEA has been shown to inhibit neutrophil migration[94] and its levels positively correlated with their phagocytic capabilities,[92] many studies consistently reported the failure of AEA to effectively inhibit superoxide and hydrogen peroxide production, so being almost inefficient in altering the microbicidal neutrophil burst reaction.[95–97] At any rate, these effects seem to be independent of cannabinoid receptors. However, CB_2 activation has been recently shown to reduce the release of metalloproteases from neutrophils, so potentially reducing vulnerability in atherosclerotic plaques.[98] Conversely, 2-AG seems to be an activator of human neutrophils, by stimulating myeloperoxidase release, leukotriene B_4 biosynthesis, kinase activation and calcium mobilization.[99] It also induces increased levels of antimicrobial effectors, thereby being a potent regulator of host defence *in vivo*.[100] As expected, these effects on neutrophil activation were not mediated by CB_2, because of the very low levels of its expression in these cells, but were rather the result of its hydrolysis and subsequent metabolism into leukotriene B_4, with activation of Leukotriene B4 receptors 1. Additional data supported a role for 2-AG in controlling RhoA activation, thereby suppressing neutrophil migration.[101] Figure 4 summarizes the main pathways of eCB signalling in neutrophils and NK cells.

Eosinophils, basophils and mast cells

These rare cell populations share similar appearance and function and are involved in allergy and anaphylaxis as well as in wound healing and in defence against pathogens. However, they differ in that they arise from different cell lines and in that eosinophils and basophils are found in the blood whereas mast cells are tissue resident (i.e. connective and mucosal tissue, nervous system).[102,103] Furthermore, eosinophils play a major role in dealing with elimination of large parasites.[103] As yet, no evidence has been reported on eCB signalling for either murine or human basophils. Very few reports have addressed eosinophil response to eCBs, and to 2-AG in particular (Fig. 5). The latter compound was found to induce the migration of human eosinophils in a CB_2-dependent manner and consistently this receptor was particularly expressed in these cells.[104] The same authors, by an ether-linked non-hydrolysable analogue of 2-AG, demonstrated that its migratory effect was attributable to chemotaxis and not to chemokinesis. Yet 2-AG potency was significantly lower than that of well-known and strong eosinophil chemoattractants, such as platelet-activating factor, RANTES and eotaxin.[105] These studies suggest that CB_2 and its endogenous ligand 2-AG may be potentially involved in allergic inflammation, accompanied by eosinophil infiltration, and this was indeed demonstrated in a mouse model of contact dermatitis.[106] A recent paper investigated the mechanisms of 2-AG-induced migration

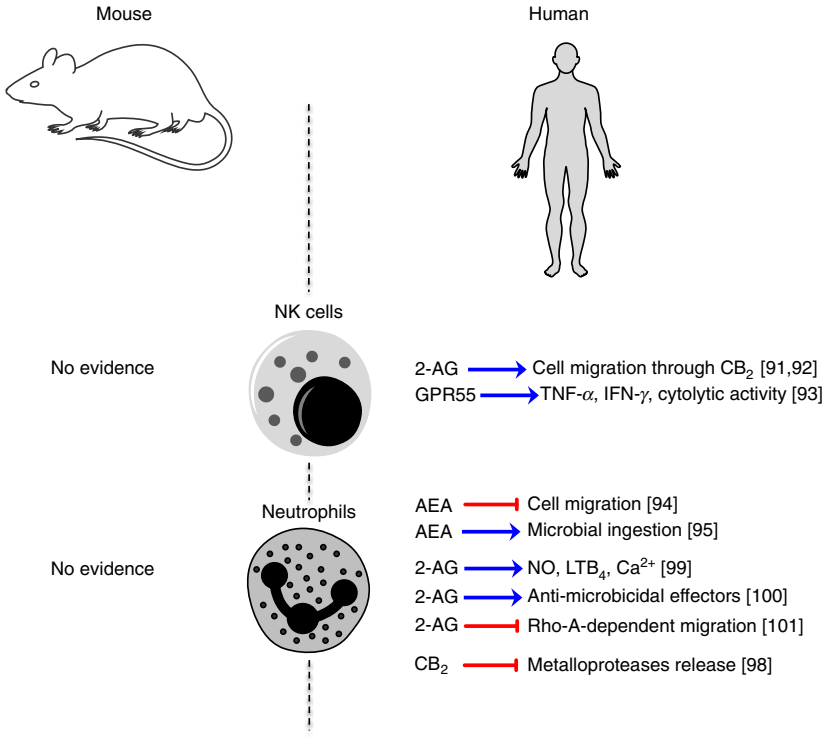

Figure 4. Schematic representation of endocannabinoid (eCB) signalling in murine and human natural killer cells and neutrophils.

of human eosinophils, confirming that this eCB in combination with IL-5 has the ability to activate and modulate eosinophil functional responses, and that the 15-lipoxygenase pathway is very probably involved in the regulation of these activities.[107] Of note, the most studied eCB in allergy is PEA. Indeed, this substance has been extensively investigated in mast cells (especially in wild-type rats) that produce high levels of PEA and express both CB_1 and CB_2.[108–110] On murine mast cells, PEA is a strong inhibitor of mast cell degranulation and activation,[111] also contributing to reduce the severity of spinal cord trauma.[112] Interestingly, a recent work hypothesized that the anti-nociceptive role of PEA in inducing relief in neuropathic pain correlates with its modulation of these non-neuronal cells.[113] Also AEA has been shown to inhibit mast cell degranulation in a human mast cell line, where this lipid was effectively degraded through a nitric oxide-sensitive endocannabinoid membrane transporter and FAAH.[114] This was confirmed 10 years later, demonstrating that AEA limits excessive mast cell maturation and activation in a CB_1-dependent mechanism in a human hair follicle organ culture model, suggesting that normal skin mast cells are indeed modulated by the ECS.[115] The involvement of AEA and CB_1 in modulating human mast cell functions was further confirmed by the observation that in human airway mucosal mast cells, maturation and excessive activation were inhibited by the endocannabinoid tone through CB_1 stimulation.[116] A very recent and interesting work further unravelled the biological implication of AEA-CB_1-mediated mast cell modulation in mast cell-deficient mice, showing that AEA activation of CB_1 in mast cells induced monocyte chemotactic protein-1-mediated recruitment of monocytic and anti-inflammatory myeloid-derived suppressor cells.[117] The main effects of PEA and AEA on murine and human mast cells are summarized in Fig. 5.

Endocannabinoid signalling in adaptive immunity

T lymphocytes

T lymphocytes (or T cells) play a central role in cell-mediated immunity, and comprise several subsets, each with a distinct function, including $CD4^+$ T helper cells, $CD8^+$ cytotoxic T cells, memory T cells, regulatory T cells and mucosal-associated invariant T cells.[118] The first evidence for an immunosuppressive role of eCBs on T cells came as early as 2 years after the isolation and purification of AEA, demonstrating its dose-dependent anti-proliferative effects on human T cells. Indeed, micromolar doses of AEA rapidly inhibited mitogen-induced DNA synthesis, and this was associated with induction of apoptotic cell death.[119] Since then, interest was primarily focused on phytocannabinoids and synthetic agonists/antagonists selective for CB_1 or CB_2. Only after more than 10 years did study of the immunomodulatory properties of eCBs on T cells begin to flourish – especially for AEA, which is the most studied eCB compared with 2-AG or PEA,

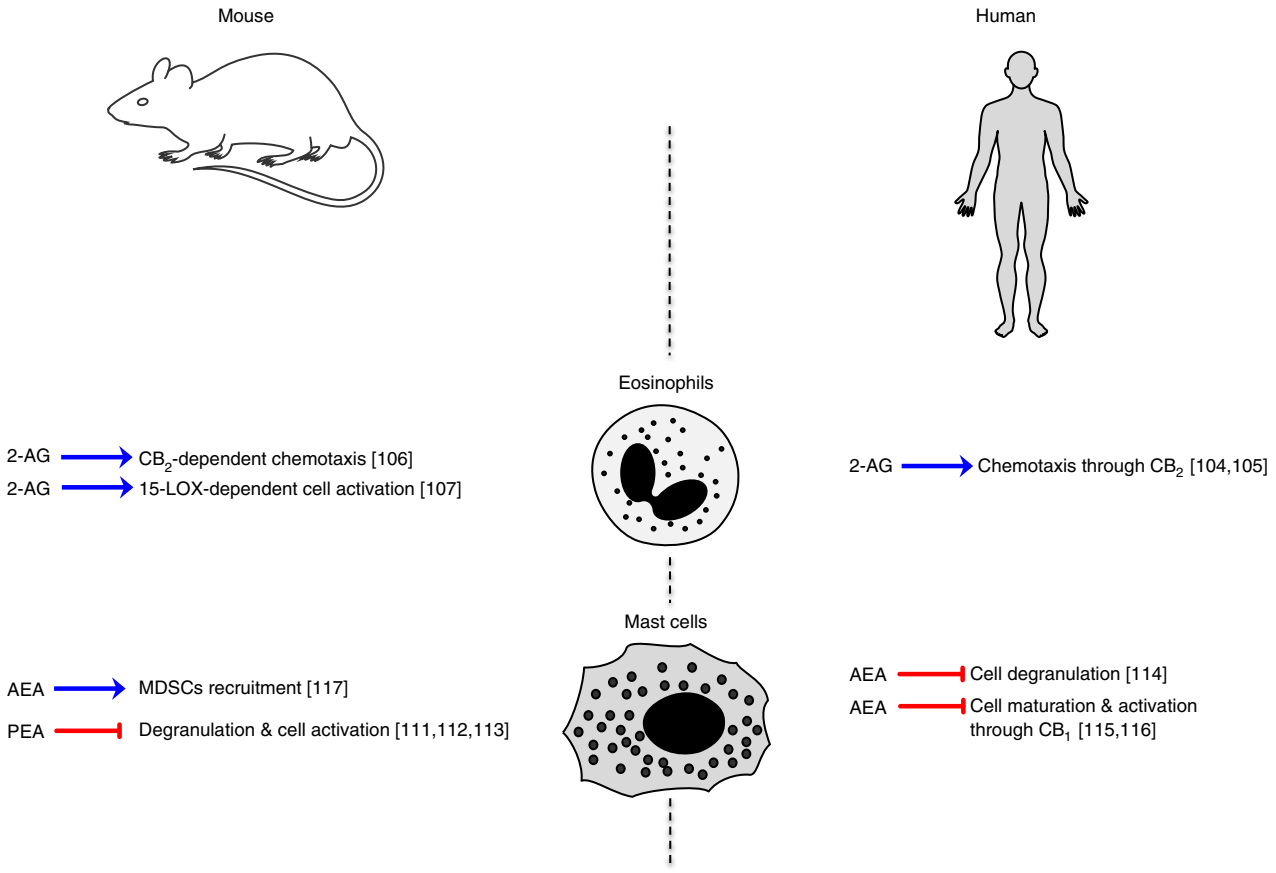

Figure 5. Schematic representation of endocannabinoid (eCB) signalling in murine and human eosinophils and mast cells.

which received little investigation. It is now accepted that AEA is a potent immunosuppressor of T-cell proliferation and cytokine release, acting mainly through CB_2 and PPAR-γ and most likely through nuclear factor-κB inhibition. This pathway has been largely investigated in mouse T cells,[87,120] in the human Jurkat T-cell line[121,122] and in human peripheral T lymphocytes.[123,124] Our group was the first to demonstrate the anti-proliferative effect of AEA on both CD4 and CD8 T-cell subsets, without any effect on cell viability.[125] In addition, we disclosed its inhibitory effect on interferon-γ-producing Th1 and IL-17-producing Th17. This effect of AEA on Th17 has been recently reproduced in a mouse model of hypersensitivity, where it was also shown to be mediated by IL-10 and mitochondrial RNA induction.[120] Interestingly, cytokines have been shown to directly influence the ECS of T lymphocytes, inasmuch as the Th2 cytokines IL-4 or IL-10 had a stimulatory effect on FAAH, whereas the Th1 cytokines IL-12 and interferon-γ reduced FAAH activity and protein expression,[126] overall suggesting an eCB-triggered self-sustaining anti-inflammatory loop. In disagreement with these results, Lissoni *et al.* reported that AEA does not inhibit human T-cell proliferation and cytokine production, probably because of the presence of albumin in their *in vitro* experiments, which is known to bind AEA and so reduce its biological activity.[127] The strong involvement of CB_2 in mediating AEA anti-inflammatory effects is supported by a reduction of eCB immune modulation of T cells from a common CB_2 polymorphism,[128] and the evidence that formation of T cells requires this receptor.[129] The anti-inflammatory role of 2-AG on T cells, instead, was shown to be independent of cannabinoid receptors, and its significant suppression of IL-2 expression in Jurkat T cells was mediated by a COX-2 metabolite of 2-AG, probably by activating the PPAR-γ.[122,130]

B lymphocytes

B lymphocytes (or B cells) are involved in the production of antibodies against antigens (humoral immunity), but they are also capable of acting as antigen-presenting cells.[131] Antibody-producing plasma cells are among the immune cells that express the highest levels of CB_2, with human B cells expressing one transcript and mouse B cells expressing three transcripts, specifically selected during B-cell activation by lipopolysaccharide.[132] However, most of the research has focused only on the use of

phytocannabinoids and 'syntho-cannabinoids', rather than on eCBs, trying to understand the functional role of this receptor in B cells. Indeed, CB_2 was identified as a crucial receptor for mouse B-cell differentiation at the end of the 1990s, as it was markedly expressed in mantle zones of secondary follicles and less in germinal centres, and its expression was down-regulated during B-cell differentiation.[133] Furthermore, CB_2 was found to be essential also for mouse B-cell subset formation,[129] and for retention of immature B cells in bone marrow sinusoids[134] and in splenic marginal zones.[135] CB_2 was also reported to mediate immunoglobulin class switching from IgM to IgE,[136] suggesting that this cannabinoid receptor could have a crucial role in the generation of B-cell repertoire and the regulation of Th2-type humoral responses. Only two works investigated the role of eCBs, in particular of 2-AG, in mouse B-cell functions, showing that this bioactive lipid induced migration of $B220^+$ $CD19^+$ B cells,[48] preferentially by attracting unstimulated naive B cells rather than activated and/or class-switched germinal centre B cells in a CB_2-dependent manner.[137] Surprisingly, no evidence of eCB signalling on human B cells has been gathered. Furthermore, it is yet to be explored whether the effects on mouse B cells are direct or are indirectly induced through other immune cells (like T cells and macrophages), required for B-cell activation. Figure 6 summarizes the principal effects on both T and B lymphocytes.

Concluding remarks

Research on the ECS on the immune system strongly suggests that its lipid mediators and their receptors exert pleiotropic and complex immunoregulatory effects. The generally immunosuppressive role of classical eCBs (AEA and 2-AG) on the different immune cell populations is abundant compared with their congeners (PEA) and is reasonably equivalent in both mice and humans, making them 'master regulators' of the innate-adaptive immune axis. Although some immune cells can respond to the different eCBs, it seems that their effects are strictly dependent on cell type; for instance, T cells mainly respond to

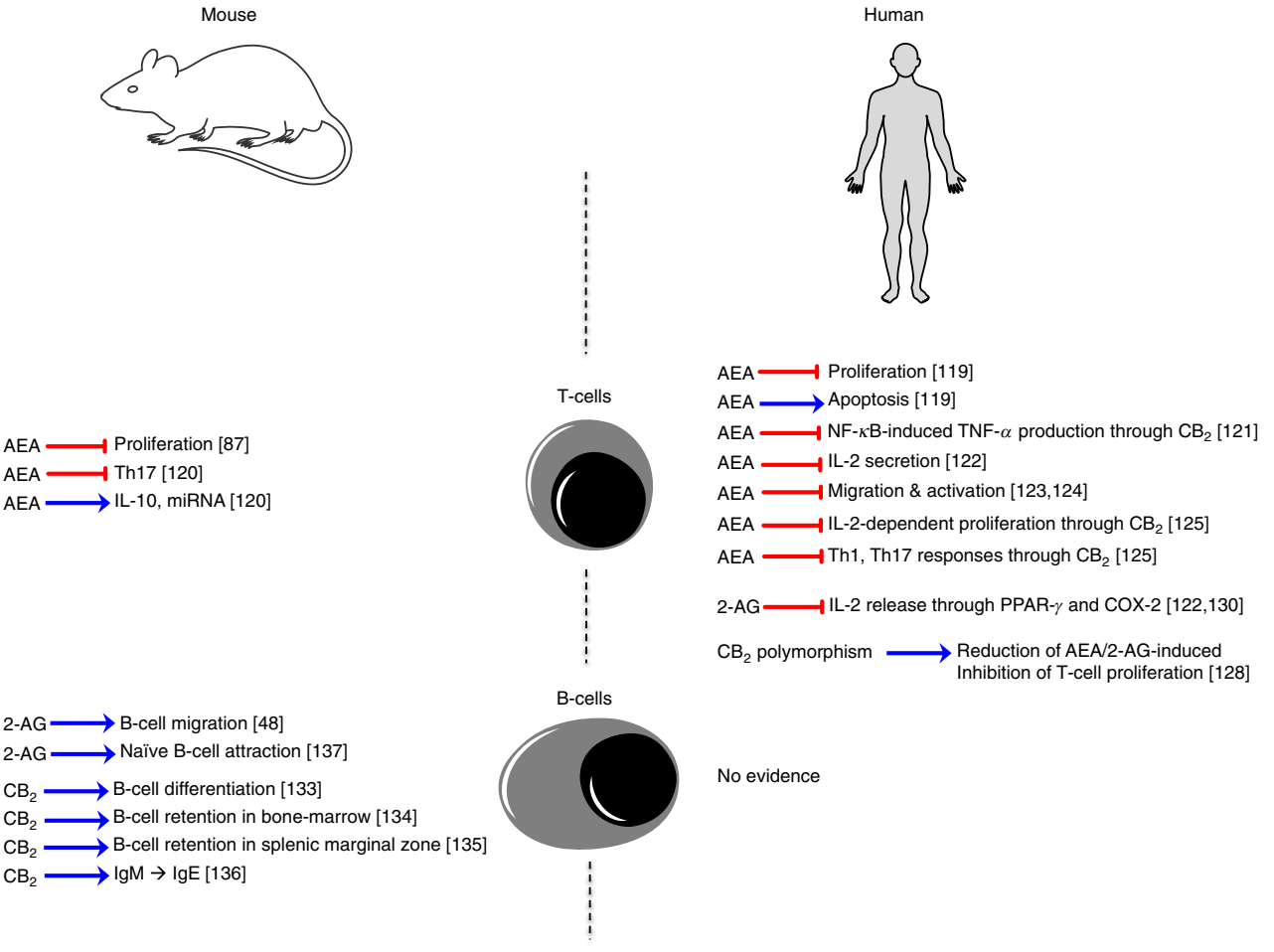

Figure 6. Schematic representation of endocannabinoid (eCB) signalling in murine and human T and B lymphocytes.

AEA, whereas eosinophils respond to 2-AG. Moreover, not only have the effects of eCBs been reported for some immune cells (NK cells, neutrophils and B cells) on mouse or human cell populations, but also some immune cells (regulatory T cells, $\gamma\delta$ T cells, or mucosal-associated invariant T cells) have never been investigated and neither have the subpopulations of each innate or immune cell type. Although the immunomodulatory effects of eCBs mainly result from either *in vitro* or *ex vivo* studies, their corresponding functions *in vivo* require further confirmation and need to be fully elucidated, along with their underlying molecular mechanisms. Although these studies support the proposition that the CB_2 receptor may represent a novel pharmacological target for selective agonists designed to suppress autoreactive immune responses while avoiding CB_1 receptor-dependent psychoactive adverse effects, it seems that the modulation of the endocannabinoid levels by specifically inhibiting their breakdown enzymes (such as FAAH) or by inducing their production can provide a new avenue of research in the regulation of immune responses. On this basis and also considering the similar effects of eCBs in both mice and humans, autoimmune models of disease represent a valuable setting in which to study the pharmacological modulation of the ECS, especially in the light of the fact that the vast majority of immunomodulatory/immunosuppressant drugs available to clinicians for the treatment of several autoimmune diseases carry as a side effect the occurrence of infective diseases. In this context, as far as the eCB signalling is concerned, the risk of overlooking aspects of human immunology that cannot be modelled in mice, so precluding a translation into human clinical trials, seems to be minimal, yet calls for caution. Hence improvements in studies of the pathophysiological functions of eCB signalling and its modulation will help to translate this knowledge into the clinical setting to develop new immunomodulatory therapies or refine existing ones.

Acknowledgements

We wish to thank Professor Giorgio Bernardi for his continuing support of our studies on immunoregulation of endocannabinoid signalling and Dr Giovanna Borsellino for helpful discussion and her precious contribution in the final editing of the manuscript. Financial support from Ministero dell'Istruzione, dell'Università e della Ricerca (PRIN 2011 grant) to MM, from the Ministero della Salute (RF-2011-02346771) to LB and from Fondazione Italiana Sclerosi Multipla (FISM grant 2013/R/8) to VC and (FISM grant 2013/R/2) to LB is gratefully acknowledged.

Disclosures

The authors declare no competing interests.

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